Prokazyme

Product number: Gtfa163
Lot number: 402-01

ThermoActive™ Glucansucrase

Product information

Product Gtfa163: 100 µl enzyme solution containing 1 mg/ml enzyme in a storage buffer (20 mM Tris-HCl pH 8.0, 10 mM $CaCl_2$ and 50% glycerol). Store at -20°C.

Enzyme activity

The enzyme transfers glucose from sucrose to make a highly branched, high-molecular-weight α-D-glucan with (1→4) glucosidic linkages but also some (1→6)-linked glucosyl units and 4,6-disubstituted glucosyl units at the branching points.

Assay and Unit definition

Glucose transferase activity was determined at 50°C for 60 hours in a 25 mM sodium acetate buffer at pH 4.7, containing 1 mM $CaCl_2$ and 100 mM sucrose. This gave complete utilization of the sucrose with about 75% going into α-D-glucan.
One unit (U) of enzyme activity is the amount that leads to the release of 1 µmol of fructose from sucrose per minute.

Exopolysaccharide synthesis:

The enzyme produces the α-D-glucan polymer (MW $3.5x10^6$ Da) with a composite structure, that includes all identified structural elements, as shown in Fig. 1.

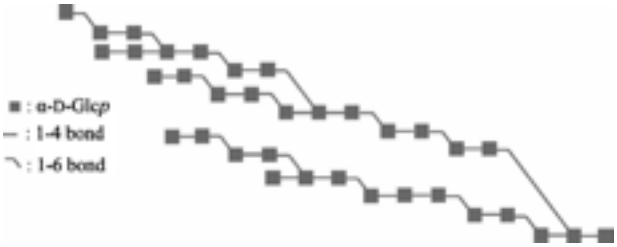

Figure 1: Schematic representation of product (1)

Oligosaccharides from sucrose:

With only sucrose present, and before branching starts, the enzyme activity will yield a linear oligosaccharides via alternating α(1→4) and α(1→6) linkages. Malto-oligosaccharides (DP2-DP6) in the absence of sucrose are slow substrates. With high concentration of sucrose the formation of low-molecular-mass oligosaccharides was favoured over high-molecular-mass polysaccharides.

Research & Development

This product is made in cooperation with Prof. Dr. L. Dijkhuizen, University of Groningen, The Netherlands.

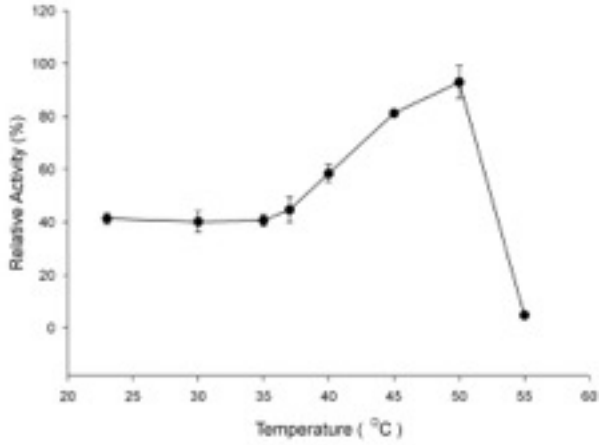

Figure 2: Temperature profile of the enzyme activity (2)

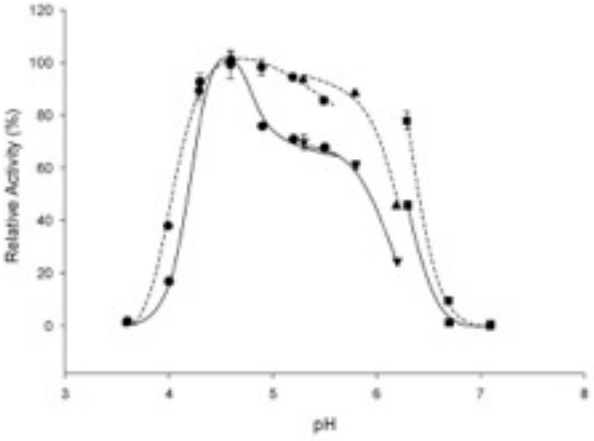

Figure 3: pH profile of the enzyme activity. Solid line, transferase activity; dashed line, hydrolysis activity (2).

References

1) Van Leeuwen SS, Kralj S, van Geel-Schutten IH, Gerwig GJ, Dijkhuizen L, Kamerling JP (2008) Structural analysis of the α-D-glucan (EPS35-5) produced by the Lactobacillus reuteri strain 35-5 glucansucrase GTFA enzyme. *Carbohydr. Res*. **343**: 1251–1265.

2) Kralj S, van Geel-Schutten GH, van der Maarel MJEC & Dijkhuizen L (2004) Biochemical and molecular characterization of Lactobacillus reuteri 121 reuteransucrase. *Microbiology* **150**: 2099–2112.

3) Leemhuis H, Pijning T, Dobruchowska JM, van Leeuwen SS, Kralj S, Dijkstra BW, Dijkhuizen L. (2013) Glucansucrases: Three-dimensional structures, reactions, mechanism, α-glucan analysis and their implications in biotechnology and food applications. *J. Biotechnol.* **163**:250-272.

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